Journal of Pharmaceutical and Health Sciences 2017;5(3), 207-216.

Original Article

JPHS

Pharmacognostic Study on Argyreia pilosa Wight & Arn. Root

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Abstract

Background: Ethnomedicinally, the plant Argyreia pilosa Wight & Arn. (Convolvulaceae) has long been utilized in various disorders in the conventional system; most significantly it is utilized against sexually transmitted diseases, skin troubles, diabetes, rheumatism, cough, and quinsy. The key challenge experienced in the standardization of herbal drugs is the lack of proper identification of plant source. Therefore there is certainly have to establish quality control parameters by utilizing pharmacognostic and phytochemical evaluation, that ensure the purity, safety, and efficacy of medicinal plant A. pilosa.
Aim: To assess pharmacognostic characteristics which include macroscopic, microscopic and physicochemical parameters of the root of A. pilosa.
Methods: Micro and Macroscopic characters of fresh and dried root samples were investigated. Physicochemical parameters had been done by using WHO recommended parameters, preliminary phytochemical and fluorescent analysis of root sample were carried out for proper identification and standardization of root of A. pilosa.
Results: The color, shape, size, odor, and surface characteristics were noted from the root and powdered root material of A. pilosa. Light electron microscope i.e., Olympus CX-21i trinocular Microscope images of cross section of root and powdered root revealed that the presence of cork cells, Xylem fibers with tapered ends, lignified xylem vessels, phloem fibers, medullary rays, sclerides and parenchymatous cells. Phytochemical screening showed the presence of flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins. Physicochemical parameters such as moisture content, ash value, extractive value and fluorescent behavior of root powder were determined. These parameters are helpful to differentiate the powdered drug material. Conclusion: The current research is useful in order to supplement the information with regard to its standardization, identification and in carrying out further investigation in Ayurvedic system of medicine.

Keywords: Pharmacognostic, Phytochemical, Argyreia pilosa Wight &Arn., Physicochemical, and fluorescence studies.

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INTRODUCTION

Medicinal plants tend to be playing a crucial role in conventional medicines for remedy of different health problems. On the other hand, a vital barrier that has obstructed the promotion in the utilization of alternative medicines in the developed nations is no proof of documentation and lack of stringent quality control measures. There is also a requirement for the records of all the research work meted out on conventional medicines by means of documentation. With this particular problem, it has become essential to make assurance regarding the standardization of the plant and its parts to be utilized as a medicine. In the process of standardization, we can make use of various techniques and methodology to attain our objective in a stepwise manner e.g. pharmacognostic and phytochemical studies. These methods and procedures are useful in identification and standardization of the plant material. Proper characterization and quality assurance of beginning material is an important step to make sure a reproducible quality of herbal medicine to help us to rationalize its safety and efficacy. For this reason, we have carried out pharmacognostic studies of Argyreia pilosa (A. pilosa) belongs to family Convolvulaceae [1]. This kind of study will not only assist in authentication but also assures reproducibility of herbal products in marketing [2].

In the current study, we are emphasizing our investigation on one of the commonly available plant in India i.e., A. pilosa, belongs to family Convolvulaceae. The family Convolvulaceae contains nearly 1650 predominantly exotic species. The genus Argyreia, with around 135 species, some of the important species include A. aggregate, A.cuneata, A. cymosa, A. daltoni, A. elliptica, A. fulgens, A. kleiniana, A. malabarica, A. nervosa, A. pilosa, A. setosa, A. strigosa and A. speciosa [3-5]. A. pilosa is a Twiner, branchlets are reddish and hirsute; leaves are simple, alternate, broadly ovate, 7 – 10 X 7 – 9 cm, acute apex, subcordate base, entire margin and nerves are prominent up to 7 - 8 pairs. Flowers are pink, in axillary, capitates heads, peduncle long 2-3 cm, bracts linear, bristly hair to 1cm long, calyx 5 lobed, lobes unequal, nearly free to base, oblong – lanceolate to 0.8cm long, corolla infundibular, to 4 cm, lobes spreading, stamens included. Fruit berry [6,7].

A. pilosa is an ornamental, in addition to a medicinal plant. All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by the Indian traditional healer. Its root is utilized to cure a various illness like sexually transmitted diseases viz., gonorrhea and syphilis, blood diseases. Traditionally, the paste of the leaves is applied to the neck region for a cough, quinsy and applied externally in case of itch, eczema and other skin troubles, antidiabetic, antiphlogistic, rheumatism, reduce burning sensation and antidiabetic [6, 7]. Young wines are mixed together with rhizome of ginger are spread all around the body to relieve from fever. The decoction of its root used to treat diarrhea and cathartic [8, 9].A vast range of phytochemical constituents has been separated from the genus Argyreia i.e., glycosides, alkaloids, amino acids, proteins, flavonoids, triterpene and steroids [10]. The genus Argyreia has been reported various biological activities including nootropic, aphrodisiac, antioxidant, antiulcer, immunomodulatory, hepatoprotective, anti-inflammatory, antihyperglycaemic, antidiarrheal, antimicrobial, antiviral, nematicidal, anticonvulsant, analgesic, anti-inflammatory, wound healing and central nervous depressant activities [10-14]. Even though the drug has many uses, it's pharmacological and phytochemistry is very poorly explored [15].

Therefore the current investigation had been carried out to study the morphological, microscopical, physicochemical and phytochemical evaluation along of root of A. pilosa with the purpose of contributing to the establishment of monograph [16, 17].

MATERIAL AND METHODS

Collection and Authentication of Plant material

The plant material was obtained from Tirupati, Chittoor district of Andhra Pradesh, India during the month of March 2016 and authenticated by Dr. K. Madhava chetty, Taxonomist, Sri Venkateswara University Tirupati, India. Voucher specimen No. 1922 was deposited at the herbarium for future reference. One portion of the root is preserved in Formalin (5ml): Acetic acid (5ml): 70% Alcohol (90ml) mixture for histological studies and the remaining portion was shade dried, powdered and sieved through 20 mesh and kept in an air tight container for future use [18, 19].

Chemicals

All analytical grade chemicals were utilized in this study were procured from E. Merck, Germany. absolute alcohol, Phloroglucinol, acetic acid, chloral hydrate, H2SO4, NaOH, HNO3, FeCl3, distilled water, Conc. HCl and chloroform.

Pharmacognostic evaluation

Organoleptic evaluation

Organoleptic evaluation of A. pilosa root has been carried out in accordance the color, size, odor, shape, taste, surface and fracture as per WHO Quality Control methods of herbal medicine [20].

Microscopic evaluation

Preparation of sections

The root bark was placed in a test tube containing sufficient water and was boiled for few minutes. The softened bark was transversally and longitudinally sliced into fine sections. Microscopic studies had been done by preparing thin hand section of root with the help of sharp cutting edge of the blade, then cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerin. The model of microscope used for the study of different characters was Olympus CX-21i trinocular Microscope, illumination halogen.

Powdered Microscopy

The powder microscopy was carried out in accordance with the procedure described in Khandelwal [21].

Preparation of extracts and preliminary phytochemical analysis

The powdered material had been extracted with various solvents according to its polarity i.e., petroleum ether, ethyl acetate, chloroform, and methanol. 100g root powder was extracted with 500 ml of the respective solvent by maceration at room temperature for 24 hours. Then, filtered through Whatman filter paper and collect the filtrate, concentrated with roto-evaporator. Then, the extracts had been subjected to preliminary phytochemical screening according to standard methods [21-23].

Physicochemical Analysis

Physicochemical parameters such as ash value, moisture content and extractive values were determined according to the procedures mentioned in WHO quality control methods for herbal materials as follows [20].

Determination of Loss on Drying

About 10 g of powdered drug (without preliminary drying) had been weighed and placed in a tared evaporating dish and was dried up at 105°C. The drying and weighing were being carried out at 1 h intervals till the variance between two successive weighings had not been more than 0.25%. A consistent weight was supposed to have reached when two successive weighing after drying for 30 min and cooling for 30 min in a desiccator, showed not more than 0.01 g difference [24, 25].

Determination of Total ash

About 2.0 g of the powdered drug had been weighed accurately and incinerated in a silica

crucible at a temperature not exceeding beyond 450°C until free from carbon. The resulting ash had been cooled and then weighed. The process was recurring to obtain a constant weight. The percentage of total ash with reference to the air-dried drug was finally calculated [24, 25].

Water Soluble ash

The ash had been obtained according to the method defined above and boiled for 5 minutes along with 25 ml of water, filtered and the insoluble matter had been obtained on an ash less filter paper. It was additionally washed along with hot water and inflamed for about 15 minutes at a temperature not exceeding beyond 450°C and weighed. The difference in weight of inflamed and total ash represents the water-soluble ash. The percentage of water soluble ash was determined with reference to the air dried drug [24, 25].

Determination of Acid Insoluble ash

The ash was obtained according to the method defined above and boiled for 5 minutes with 25 ml of 2 M hydrochloric acid, filtered and insoluble matter was collected on an ash less filter paper. It was further washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug [24, 25].

Determination of extractive value

Water soluble extractive value

Four gm of the air dried coarsely powdered drug had been macerated with 100 ml of water in a closed flask for 24 hours, and shaken repeatedly during first 6 hours then allowed to stand for 18 hours. It was filtered; 25 ml of filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drug [24, 25].

Alcohol soluble extractive

Four gm of the air dried coarsely powdered drug was macerated with 100ml of ethanol in a closed flask for 24 hours, and shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, during filtration, precaution was taken against loss of ethanol; 25 ml of filtrate was evaporated in a flat shallow dish, and dried at 105°and weighed. Percentage of Ethanol soluble extractive value was calculated with reference to air-dried drug [24, 25].

Fluorescence analysis

Various reagents were utilized to check the fluorescence activity. In this, 0.1 g of plant powder was blended with 1.5 ml of respective reagent. The mixture was placed on the slide for a minute and observed under visible light, short ultra-violet light (254 nm) and long ultraviolet light (365 nm) [18].

Figure. 1: Macroscopic Characteristics of Argyreia pilosa Wight & Arn. Root

Table 1: Organoleptic characteristics of Argyreia pilosa Wight & Arn. Root

Organoleptic characters	Observation
Colour	Buff
Odour	Characteristic
Taste	No taste
Size	25 to 30 cm
Texture	Rough
Fracture	Fibrous

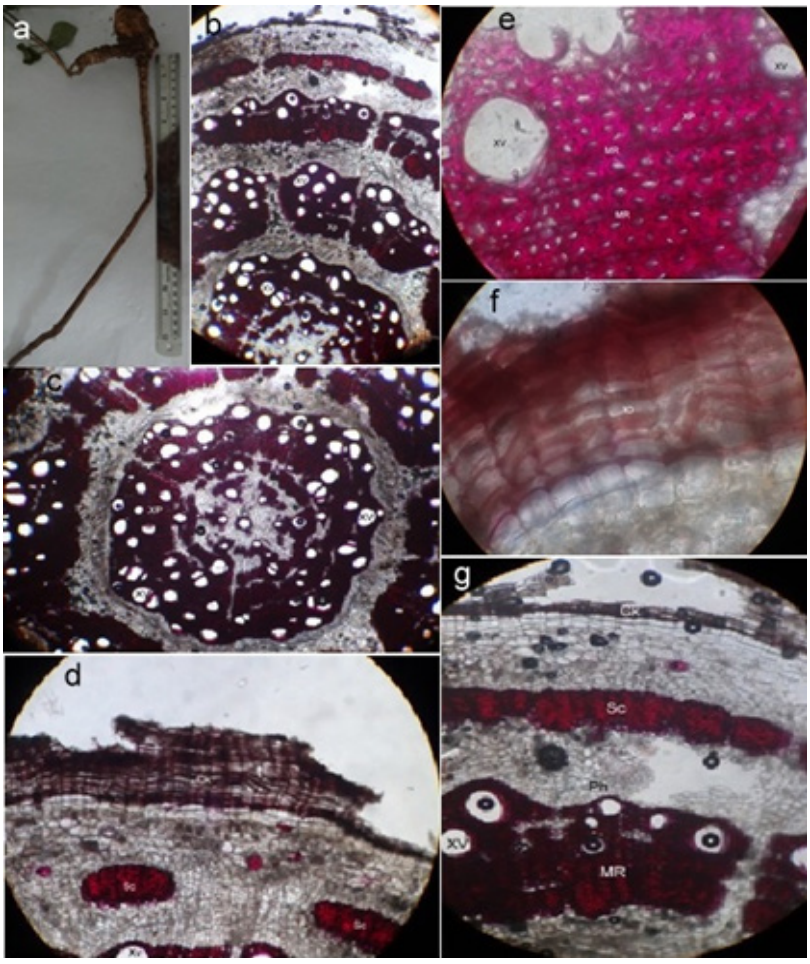

Figure 2: T. S of Root of Argyreia pilosa Wight & Arn. a. Morphology of root, b. T. S of root entire view, c. T. S of root showed the centre portion of root d. T. S of root showed cork & Scleroidal cells, e. T. S of root showed medullary rays and Xylem Vessels, f. Magnified (40X) view of cork, g. T. S of root in Magnified (40X) view. Abbreviations: Ck: Cork Cells, Sc: Scleroidal cells, XV: Xylem Vessels, MR: Medullary rays, Ph: Phloem

RESULTS

Organoleptic evaluation

Root powder was buff in color, no characteristic odor, and taste (Fig. 1, Table 1).

Microscopical Evaluation

Root

Transverse section of root revealed the presence of cork, composed of about 10-12 tangentially

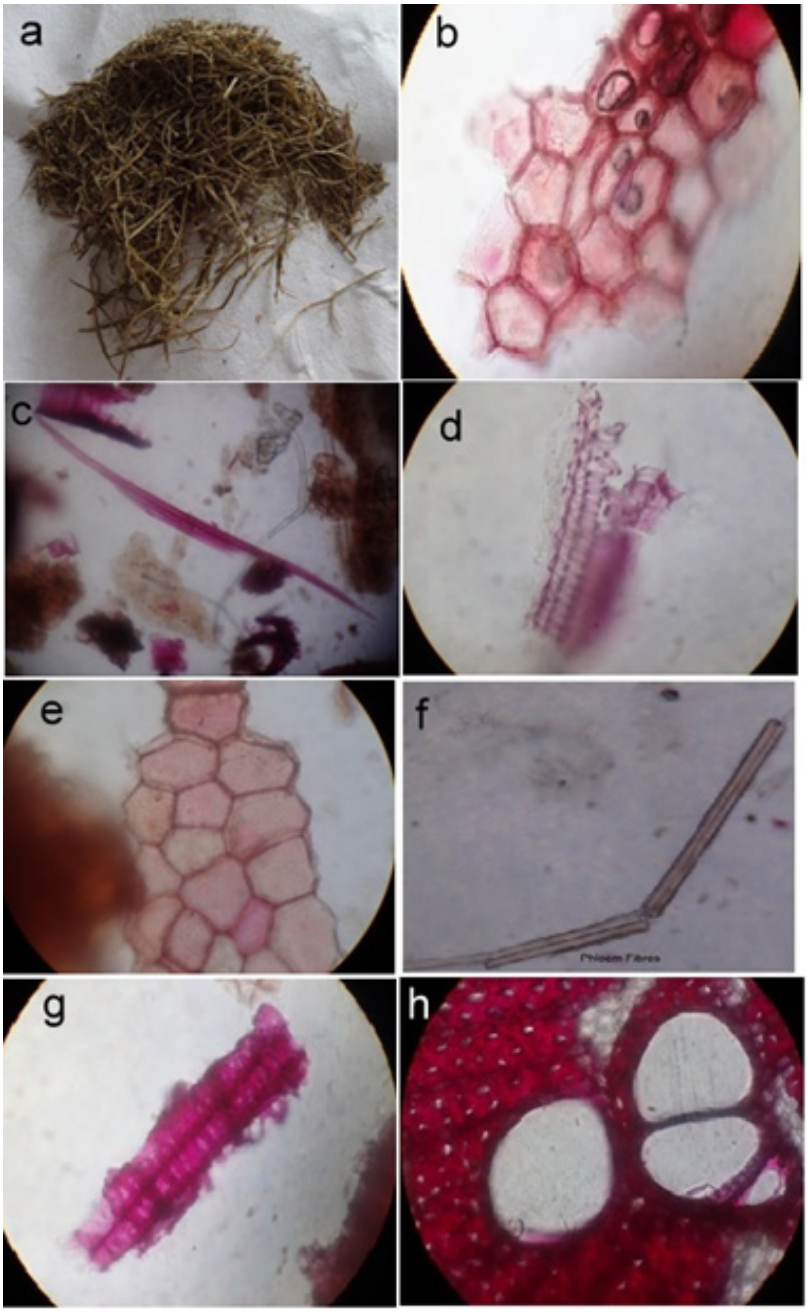

Fig. 3: Powder Microscopy of the root of Argyreia pilosa Wight & Arn. a. Root powder, b. Cork cells in surface view, c. Xylem fiber with tapered ends, d. Lignified Xylem vessels, e. Parenchyma cells, f. Phloem fibers, g. Medullary rays, h. Xylem vessels with medullary rays.

elongated, thick walled cells. Beneath, there is a presence of stone cells, which are lignified in nature. Cortex was comprised of thin walled parenchymatous cells having very small intercellular spaces. The endodermis revealed the presence of phloem and xylem, which were very well developed. Xylem occupies more region than the phloem in endodermis. The phloem is present in between the medullary rays. The medullary rays are usually parenchymatous and they are uniseriate. Phloem revealed the presence of phloem fibers which are non-lignified in nature. Additionally, it revealed the presence of phloem parenchyma. The xylem occupies the whole central region and was also surrounded by uniseriate medullary rays. Xylem tissue comprises of spiral xylem vessels, xylem fibers, and xylem parenchyma as shown in Fig. 2.

Powder Microscopy

The powdered root was brown in color, which revealed the presence of cork cells, Xylem fibers, Xylem vessels, Parenchyma cells, Phloem fibers, sclerides and medullary rays as showed in the fig. 3.

Preliminary Phytochemical Analysis

The results of qualitative phytochemical analysis of crude powder of A. pilosa root was tabulated in Table 2.

Table 2: Phytochemical analysis of various extracts of Argyreia pilosa Wight & Arn. Root

Phytoconstituents	Method	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract
Flavonoids	Shinoda Test	-	+	-	+
	Zn. Hydrochloride test	-	+	-	+
	Lead acetate Test	-	+	-	+
Volatile oil	Stain test	-	-	-	-
Alkaloids	Wagner Test	-	-	+	+
	Hager's Test	-	-	+	+
Tannins & phenols	FeCl3 Test	-	-	-	+
	Potassium dichromate test	-	-	-	+
Saponins	Foaming Test	-	-	-	-
Steroids	Salkowski test	+	-	+	+
Fixed oils and fats	Spot test	+	-	-	-
Carbohydrates	Molish test	-	-	-	+
Acid compounds	Litmus test	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	+
Proteins	Biuret	-	-	-	+

"+"- Present; "-" - Absent

Fluorescence Analysis

Fluorescence analysis of root powder was performed out after treating with different solvents. Fluorescence was observed at 254 and 365 nm comparing its change of color in visible light. The observations were tabulated in Table 4 shows the variation in color.

Table 3: Physicochemical Parameters of root powder of Argyreia pilosa Wight & Arn.

Parameters	Values %w/w
Moisture content (Loss on drying)	7.36 ± 0.11
Total ash	5.36 ± 0.33
Acid insoluble ash	1.98 ± 0.08
Water soluble ash	2.62 ± 0.06
Petroleum ether soluble extractive value	0.55 ± 0.03
Chloroform soluble extractive value	1.15 ± 0.05
Ethyl acetate soluble extractive value	2.25 ± 0.03
Methanol soluble extractive value	8.12 ± 0.06
Water soluble extractive value	11.68 ± 0.05

Table 4: Fluorescence analysis of Argyreia pilosa Wight & Arn. Root powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long (366nm)
Distilled water	Buff	Buff	Dark Brown
1 N NaOH 1N Methanol	Brownish red	Brownish green	Dark Brown
1N HCl	Buff	Greenish grey	Dark greenish gray
50% HNO3	Buff	Greenish grey	Dark greenish gray
FeCl3	Buff	Yellowish violet	Bluish green
CHCl3	Buff	Yellowish grey	Dark brown
Picric acid	Brownish yellow	Yellowish green	Pale green
Water soluble extractive value	11.68 ± 0.05		

DISCUSSION

Indian systems of medicine utilize majority of the crude drugs which are of plant origin. It is important that standards need to be set down to control and check the identity of the plant and confirm its quality before use. Hence a detailed pharmacognostic assessment is an extremely an important prerequisite. In accordance with World Health Organization (WHO), the organoleptic and histological description of a medicinal plant could be the first step towards establishing its identity and purity and should be performed before to any tests tend to be undertaken [26].

A.pilosa, extensively utilized in conventional medicines has tremendous therapeutical potential due to its various biological activities. The prominent diagnostic characteristics of root showed the presence of cork cells, Xylem fibers, Parenchyma cells, Phloem fibers and medullary

rays. These characters can be utilized for standardization of drugs as well as used for preparation of plant monograph and also reduces the possibilities of adulteration when the drug is available in the powdered form. Studies of physicochemical parameters can serve as an important source to judge the purity and quality of crude drugs. Ash values are utilized to establish the quality and purity of the crude drug. It implies the existence of various impurities like carbonate, oxalate, and silicate. The water soluble ash is water soluble part of total ash, employed to calculate the amount of inorganic substances found in the drugs. The acid insoluble ash comprises mostly silica and indicates contamination with earthy matter. The moisture content of drugs might be at a minimum level in order to suppress the growth of microorganisms like bacteria, yeast or fungi during storage. The extractive values are helpful to judge the chemical constituents present in the crude drug and also assist in the evaluation of particular constituents soluble in a specific solvent. Acid insoluble ash measures the amount of silica present, especially sand. Water soluble ash is water soluble part of total ash [27-29]. The phytochemical analysis of different solvent extracts viz., chloroform, methanol, and water were analyzed and it indicates the presence of tannins, flavonoids, steroids, glycosides, volatile oil, amino acids, proteins, and alkaloids.

Since there is no pharmacognostic work on documented of this traditionally significant valued drug, the current work had been taken up with a view to lay down standards that could be helpful to establish the authentication of this medicinally important plant. Macro and micro morphological standards discussed here can be considered as identifying parameters to authenticate the drug.

CONCLUSION

Standardization of herbal drugs is very much crucial because they are produced from heterogeneous sources which could result in variations. These kinds of variations can cause spurious results in various pharmacological and phytochemical studies. Argyreia pilosa Wight & Arn. the whole plant was recognized for many therapeutical properties, therefore, the current study might be beneficial to supplement the information in respective to its identification, authentication, and standardization; no such information is available for the same till date.

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